Instructions For Use

Version: 1.0 *Ref: IFU-Human SID* *Revision date: 2023-10-27*

EasySeq™

Human DNA Sample Identification Kit

For NGS Library Prep by Reverse Complement PCR

NimaGen.

Innovators in DNA Sequencing Technologies

Product and Company Information

EasySeq™ Human DNA Sample Identification Kit

REF RC-SID096

Research Use Only

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Symbols Used on Product Labels and in Instruction For Use

Symbol	Description
	Manufacturer
	Use-by date
LOT	Lot number
REF	Reference number
	Temperature limit for storage
Σ	Contains sufficient for <*n*> tests
	Matrix code containing the reference number, lot number and use-by date

Product Description

The EasySeq™ Human DNA Sample Identification Kit is a single-tube multiplex NGS Library Preparation Kit, for genotyping an optimized panel of 40 exonic, highly variable identification SNPs in human DNA. This profile can be utilized to extract intrinsic identifiers from human exome or genome sequencing data. The kit is intended to create illumina® compatible libraries.

EasySeq™ Human DNA Sample Identification Targets			
Amelogenin X	rs4735258	rs495680	rs3826616
Amelogenin Y	rs4870723	rs9532292	rs9962023
TXLNGY	rs7465584	rs11158685	rs2228611
rs1410592	rs1381532	rs4577050	rs10373
rs2229546	rs1536928	rs17715450	rs2296241
rs10203363	rs1572983	rs1026128	rs4148973
rs2819561	rs577993	rs1037256	rs760482
rs4688963	rs10883099	rs1292053	rs2073787
rs309557	rs4617548	rs2159132	rs5930933
rs7738	rs7300444	rs1805034	rs6568050

Reverse Complement PCR Kit Contents

NimaGen Part# RC-SID096 (store at -20 °C)	Contents
EasySeq™ Human Sample ID Probe Panel (REF: PM-SID)	1x Tube (24 µL)
2x PCR Master Mix (Hot Start HiFi) (REF: MMHS096)	1x Tube (1150 µL)
Probe Dilution Buffer enhanced (REF: PDB-Enh)	1x Tube (216 µL)

Required Materials, Not Included

Description	Vendor
Index Primer Plate, dehydrated. Choose one of the 8 available EasySeq™ Unique Dual Index plates for Illumina. Available REF: IDX96-U01, IDX96-U02, IDX96-U03, IDX96-U04, IDX96-U05, IDX96-U06, IDX96-U07, IDX96-U08. **Note:** The index sequences are available from the download section on the NimaGen website.	NimaGen
Adjustable Pipette Set (P10, P20, P100, P200, P1000)	Multiple Vendors
TapeStation, Bioanalyzer Instrument, incl. consumables.	Agilent
Ethanol Absolute, Molecular Biology Grade	Multiple Vendors
AmpliClean™ or AMPure XP Bead Solution	NimaGen / Beckman Coulter
General plasticware, DNAse free (1.5 mL tubes, pipette tips etc.)	Multiple Vendors
Mini Spinner for 1.5 mL tubes and 8-well PCR strips or PCR plates	Multiple Vendors
Magnetic stand for 1.5 mL Eppendorf tubes and/or 96-wells plates	Multiple Vendors / NimaGen
Water, PCR Grade	Multiple Vendors
Qubit Fluorometer incl. High Sensitivity consumables	Thermo Fisher Scientific
Thermocycler with heated lid, (0.2 mL standard PCR tubes), compatible with semi-skirted ABI style PCR plates and option for ramp rate programming. **Note:** Kit is validated for Applied Biosystems™ Veriti™, MiniAmp™ and SimpliAmp™ Thermal Cyclers.	Multiple Vendors
NaOH Solution (2 N)	Multiple Vendors
Tris-HCl (200 mM), pH 7	Multiple Vendors
Low TE (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)	Multiple Vendors
Illumina NGS Sequencing Instrument	Illumina®
Illumina® Reagent kit (depending on the device used)	Illumina®

General Precautions

Read the Material Safety Data Sheet (MSDS) and follow the handling instructions. Adhere to good laboratory practice and wear protective eyewear, gloves and lab coat when handling both the reagents supplied in this kit and other reagents required. Wash body parts with ample amount of water immediately if they come in contact with the reagents. Seek medical help if needed.

Use a Pre-PCR environment for setting up the RC-PCR. Sample pooling, purification and library quantification should be performed in a Post-PCR environment.

Protocol

1. Thermocycling Program

Temp	Duration	Ramp Rate (from previous step)	Cycles
98 °C	1 minute	Max	1 x
98 °C	10 seconds	Max	1 x
60 °C	5 minutes	0.1 °C/sec (or 2% of Max)	
72 °C	1 minute	Max	
98 °C	10 seconds	Max	2 x
80 °C	1 second	Max	
60 °C	30 minutes	0.1 °C/sec (or 2% of Max)	
72 °C	30 seconds	Max	
98 °C	10 seconds	Max	34 x
80 °C	1 second	Max	
60 °C	1 minute	0.5 °C/sec (or 10% of Max)	
72 °C	30 seconds	Max	
12 °C	∞	Max	1 x

Heated lid at 105 °C.

Note: This protocol takes approximately 3-4 hours to complete, but may vary per thermal cycler used. When running this protocol for the first time, start the cycling program as a dummy run, to check the predicted duration of 3-4 hours.

2. Reverse Complement PCR

In a single, closed tube reaction, the target specific RC-probes are working as a template to extend the UDI primers to synthesize functional, tailed and indexed PCR primers. This will be followed by two long hybridization/extension steps of 30 minutes and subsequently a further DNA amplification of the target regions, meanwhile synthesizing more primers.

2.1 Thaw on ice:
- Human Sample ID Probe Panel (Black cap)
- Probe Dilution Buffer Enhanced (Blue cap)
- HiFi Master Mix (White cap)

Note: The HiFi Master Mix contains iso-stabilizers and may not freeze completely, even when stored at -15 °C to -25 °C. It may contain precipitates when thawed at +2 °C to +8 °C. Always ensure that the Master Mix is fully thawed and thoroughly mixed before use.

2.2. Take the IDX PCR plate of choice and break off the number of strips needed.

Note: Register the indexes used (IDX set/strip-column number and well position for each sample). Download the index details for setting up the Illumina sample sheet.

Note: Before breaking off 8-well strips, cut the seal at the breaking line with a sharp knife.

2.3. Prepare in a fresh 1.5 mL Eppendorf tube the RC-PCR mix by combining and mixing:
- 0.2 µL Human Sample ID Probe Panel per reaction (Black cap)
- 1.8 µL Probe Dilution Buffer Enhanced per reaction (Blue cap)
- 10 µL HiFi Master Mix per reaction (White cap)
- 4 µL PCR grade H_2O per reaction

Example: 24 samples + 10% extra volume*
- 5.28 µL RC-PCR Probe Panel
- 47.52 µL Probe Dilution Buffer
- 264 µL HiFi Master Mix
- 105.6 µL PCR grade H_2O

*It is recommended to allow for a 10% excess when preparing the RC-PCR mix to correct for any pipetting loss. The kit contains extra reagent to facilitate this.

2.4. Remove the seal from the PCR plate or strip(s).

2.5. Dispense 16 µL of the RC-PCR mix (from step 2.3) to each well of the plate/strip(s).

2.6. Add to each well: 4 µL of DNA solution (optimal: 20 ng total DNA input).

2.7. Close the tube strips **carefully** with the caps provided, there should be an audible click. Mix by short vortexing, followed by a quick spin. Verify that the colour of the reaction mix is homogenously pink.

2.8. Place the samples in the thermal cycler(s) and start the RC-PCR program.

After the RC-PCR, samples have been amplified and tagged with sample-specific indexes and sequencing adapters. From this point, RC-PCR product purification is performed using a magnetic bead based purification to remove primers, dimers and salts.

3. Purification

Note: Before pooling, optionally check the unpurified PCR products on agarose.

3.1. Bring the magnetic bead solution (AmpliClean™ or AMPure XP) to room temperature.

3.2. Pool 5 µL RC-PCR products from each reaction into a 1.5 mL Eppendorf tube.

3.3. Mix well and transfer 40 µL of this pool to a new 1.5 mL Eppendorf tube.

3.4. Add 60 µL Low TE buffer or molecular grade H_2O to the tube and mix well (total volume is now 100 µL).

3.5. Beads purification:

a. Vortex the beads thoroughly to resuspend.
b. Add 100 µL bead solution to the 100 µL pool (from step 3.4) and mix well immediately by pipetting up and down 5 times.
c. Incubate for 5 minutes.

On magnet:

d. Place the tube for 3 minutes on the magnet, or until the solution is fully cleared.

e. Remove and discard all liquid carefully, without disturbing the beads.
f. Add 300 µL (freshly prepared) 75% ethanol, without disturbing the beads.
g. Wait for 1 minute.

h. Repeat steps **e.**, **f.** and **g.** for a second ethanol wash step.
i. Carefully remove all liquid without leaving traces of ethanol. (Optionally: quick spin, then place the tube back on the magnet and remove the last traces of ethanol)
j. Dry with open cap for 2-3 minutes at room temperature. **Do not over-dry.**

3.6. Elution:

a. **On Magnet:** Add 50 µL Low TE buffer to the tube.
b. **Off Magnet:** Resuspend the beads by flicking, or by short vortexing.
c. **Off Magnet:** Incubate for 2 minutes.
d. **On Magnet:** Wait for 1-3 minutes, or until the solution is fully cleared.
e. **On Magnet:** Carefully bring 40 µL of the clear solution into a new 1.5 mL Eppendorf tube, making sure not to transfer any of the beads.

The libraries are now ready for a quantitative and qualitative check, followed by NGS.

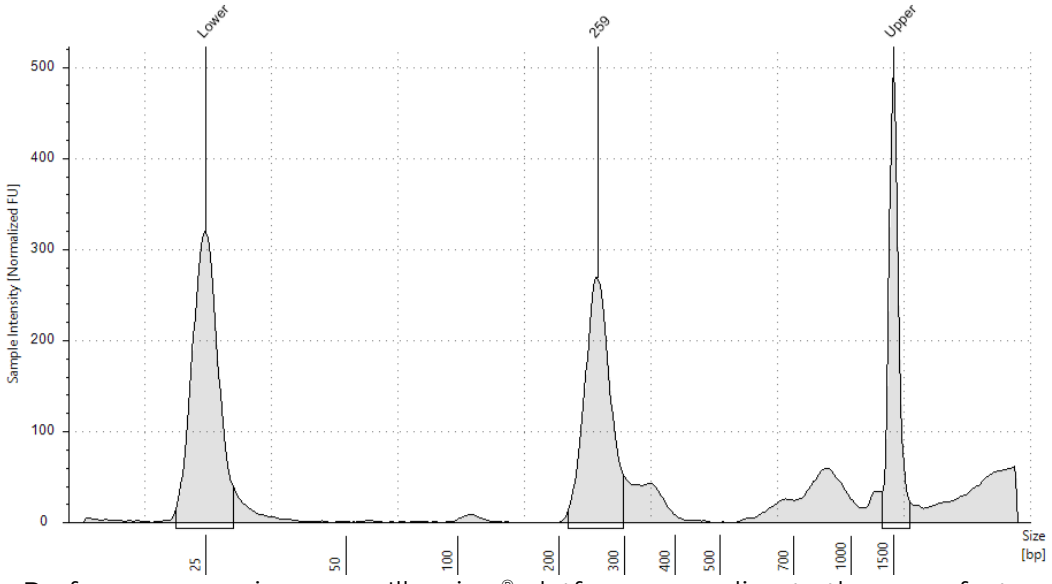
4. Sequencing

4.1. Determine the final concentration of the library or libraries by a double Qubit (HS) measurement:

- **a.** Bring the Qubit reagents to room temperature.
- **b.** Label the Qubit tubes on the lid according to the number of samples to be used plus 2 standards.
- **c.** Dilute the Qubit dsDNA HS Reagent 1:200 in Qubit dsDNA HS Buffer for each sample/ standard. It is recommended to allow for >10% excess when preparing the working solution to correct for any pipetting loss.
- **d.** For the standards: mix 190 µL of the working solution with 10 µL of the standard.
- **e.** For the samples: mix 180-199 µL of the working solution with 1-20 µL sample (total 200 µL).
- **f.** Vortex the tubes thoroughly and incubate the tubes for 2 minutes.
- **g.** Measure the standards and the samples using the 'dsDNA High Sensitivity' settings making sure to select the correct sample volume used in step **e.**.

4.2. **Optional but recommended:** Perform a qualitative verification of the library on TapeStation or Bioanalyzer, according to the manufacturer's protocol. If needed, dilute the pool. E.g. for TapeStation High Sensitivity kit, dilute to ~2 ng/µL.

Example of a clean library on TapeStation:



4.3. Perform sequencing on an Illumina® platform, according to the manufacturer's manual. Use the following guidelines:

- For calculation of the library concentrations, use an average fragment size of 250 bp.
- All targets are located within 50 bp from the start of the read, which means that a reading length of ≥75 bp is sufficient to perform the analysis of all targets.
- Required reads for single source samples for minimal 50x coverage for this panel is 5000 total reads per sample.
- Dilute the library pool to the required loading concentration for your Illumina instrument. We advise to start with a lower loading concentration for their initial sequence run and adjust in subsequent runs if needed. This avoids overclustering and potentially failure of the run.
- A spike-in of 5% PhiX is recommended for QC purposes.

Customer Support

For technical questions, assistance, or to suggest enhancements, please contact us at techsupport@nimagen.com.

Revision History

Section	Summary of changes	Version	Date
All	New document.	1.0	2023-10-26

Legal Notice

Disclaimer

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